Applicant: Mark Espy et al. Serial No.: 09/945,203

Serial No.: 09/945,203 Filed: August 31, 2001

Page



Attorney's Docket No.: 07039-247001

SPIGINALLY FILED

## VERSION WITH MARKINGS TO SHOW CHANGES MADE

## In the Specification:

A paper copy of the Sequence Listing has been added to the application.

The paragraph beginning at page 19, line 27, has been amended as follows:

The LightCycler<sup>TM</sup> instrument can amplify target nucleic acids within about 30-40 min and monitors the development of PCR product by fluorescence assay after each cycling step (amplification and hybridization). All samples were amplified by LightCycler<sup>TM</sup> PCR with primers directed to both gene 28 and gene 29. PCR primers for detection of VZV DNA using gene 28 were designed using the OLIGO program and had the following sequences: sense, 5'-GAC AAT ATC ATA TAC ATG GAA TGT G-3' (SEQ ID NO:1); antisense, 5'-GCG GTA GTA ACA GAG AAT TTC TT-3' (SEQ ID NO:2); and probes 5'-CGA AAA TCC AGA ATC GGA ACT TCT T-fluorescein-3' (SEQ ID NO:3) and 5'-Red 640-CCA TTA CAG TAA ACT TTA GGC GGT C-phosphate-3' (SEQ ID NO:4). Amplification of VZV using such primers directed toward gene 28 generated a 282 bp amplification product (Saverbrei et al., 1999, *J. Clin. Virol.*, 14:31-6). A PCR master mix (see Espy et al., 2000, *J. Clin. Microbiol.*, 38:795-9) was modified for the VZV gene 28 LightCycler<sup>TM</sup> Assay by eliminating DMSO and using 4 mM MgCl and 1 μM gene 28 primers. Samples underwent 45 cycles of: denaturation at about 95°C immediately followed by primer annealing to the template nucleic acid for about 12 secs at about 55°C, and elongation of the newly-synthesized strands at about 72°C for about 12 secs.

The paragraph beginning at page 20, line 11, has been amended as follows:

Primers and probes for detection of VZV DNA using gene 29 were designed using the OLIGO software (Molecular Biology Insights, Inc., Cascade, CO) and had the following sequences: sense, 5'-TGT CCT AGA GGA GGT TTT ATC TG-3' (SEQ ID NO:5); antisense, 5'-CAT CGT CTG TAA GAC TTA ACC AG -3' (SEQ ID NO:6); and probes 5'-GGG AAA TCG AGA AAC CAC CCT ATC CGA C-fluorescein-3' (SEQ ID NO:7) and 5'-Red 640-AA GTT CGC GGT ATA ATT GTC AGT GGC G-phosphate-3' (SEQ ID NO:8). Amplification using such gene 29 primers produced an amplification product of 202 bp. The PCR master mix (see Espy et al., 2000, *J. Clin. Microbiol.*, 38:795-9) was modified for the VZV gene 29

Applicant: Mark Espy et al. Serial No.: 09/945,203

Filed : August 31, 2001





Attorney's Docket No.: 07039-247001

LightCycler<sup>TM</sup> Assay by using 4 mM MgCl, 1  $\mu$ M gene 29 primers and 3% dimethylsulfoxide. The thermocycling program for gene 29 was the same as described above for gene 28.

